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The role of the *N*-methyl-D-aspartate receptor in the proliferation of adult hippocampal neural stem and precursor cells

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New neurons are continuously generated from resident pools of neural stem and precursor cells (NSPCs) in the adult brain. There are multiple pathways through which adult neurogenesis is regulated, and here we review the role of the *N*-methyl-D-aspartate receptor (NMDAR) in regulating the proliferation of NSPCs in the adult hippocampus. Hippocampal-dependent learning tasks, enriched environments, running, and activity-dependent synaptic plasticity, all potently up-regulate hippocampal NSPC proliferation. We first consider the requirement of the NMDAR in activity-dependent synaptic plasticity, and the role the induction of synaptic plasticity has in regulating NSPCs and newborn neurons. We address how specific NMDAR agonists and antagonists modulate proliferation, both *in vivo* and *in vitro*, and then review the evidence supporting the hypothesis that NMDARs are present on NSPCs. We believe it is important to understand the mechanisms underlying the activation of adult neurogenesis, given the potential that endogenous stem cell populations have for repopulating the hippocampus with functional new neurons. In conditions such as age-related memory decline, neurodegeneration and psychiatric disease, mature neurons are lost or become defective; as such, stimulating adult neurogenesis may provide a therapeutic strategy to overcome these conditions.

adult neurogenesis, stem cell, precursor cell, NMDA receptor, hippocampus, synaptic plasticity, long-term potentiation (LTP)

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The adult brain continuously generates new neurons throughout life. These adult-born neurons arise from endogenous neural stem and precursor cells (NSPCs), which primarily reside within two neurogenic niches: the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus, and the subventricular zone (SVZ) of the lateral ventricles. In the 20 years since adult mammalian neurogenesis started receiving substantial attention [1,2], scientists have made great progress in understanding the mechanisms that regulate stem cell maintenance, proliferation, differentiation, maturation and integration [3,4]. Early on, extrinsic cues from the environment, such as learning [5], physical activity [6], and enriched environments [7,8], were found to potently enhance hippocampal neurogenesis. It was not long before a link was made to synaptic plasticity [9], which is also enhanced by physical exercise [10], and is widely accepted as the synaptic mechanism underlying hippocampal learning and memory [11].

Synaptic plasticity and adult hippocampal neurogenesis are intimately linked, as long-term potentiation (LTP), an activity-dependent change in synaptic efficacy, can influence the activation and proliferation of NSPCs in the DG (e.g., [12]), as well as the survival of newborn neurons (e.g., [13]). These newborn neurons have biophysical characteris-

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tics that allow them to preferentially contribute back to synaptic plasticity, in that they have a lower threshold for the induction of LTP compared to mature neurons, due to their higher input resistance and slower membrane time constant [14,15]. Newborn neurons are also important for the induction of bidirectional hippocampal synaptic plasticity [16]. Furthermore, evidence is mounting to support the idea that adult-born neurons are integral participants in hippocampal-dependent cognitive processes, such as learning and memory formation [17–19], and mood regulation [20–22]. In this review, we consider the role of activity-dependent synaptic plasticity in regulating the activation of NSPC proliferation in the adult rodent hippocampus. Given that this form of plasticity requires the *N*-methyl-D-aspartate receptor (NMDAR), we also review the specific role the NMDAR has in this process.

1 Adult hippocampal neurogenesis

NSPCs have the remarkable ability to self-renew, divide and differentiate into a diverse range of functional cell types. The discovery of a quiescent stem cell population in the adult hippocampus [23] has important ramifications for the use of neurogenesis as a therapeutic strategy for functional recovery following brain injury and disease [24]. Accordingly, an intense effort has gone into understanding the mechanistic regulation of adult neurogenesis.

The production of new neurons in the adult hippocampus is a tightly orchestrated, multi-step process involving the maintenance, activation and proliferation of stem cells, differentiation and migration of intermediate progenitors, and maturation and integration of newborn neurons [25–27]. Under basal conditions, neural stem cells can either be in a resting, quiescent state, or they can be actively dividing [23,28,29]. Proliferating stem cells are capable of continuous self-renewal through mitotic cell division, and can differentiate into a number of different cell types, such as neurons, astrocytes and oligodendrocytes.

The identity of a cell within the neurogenic pathway (Figure 1) can be ascertained by examining its morphological characteristics as well as the expression of stem cell-specific markers, like glial fibrillary acidic protein (GFAP), hairy and enhancer of split 5 (Hes5), nestin, and SRY-related HMG-box gene 2 (Sox2) [30]. Progenitor cells produced from the division of these stem cells exhibit a more limited potential for self-renewal [31]; however, they are still capable of proliferation. Like stem cells, early progenitors also express the stem cell markers, Sox2 and nestin, but do not express GFAP or Hes5. Late progenitor cells start expressing the immature neuronal marker, doublecortin (DCX), and the neuronal fate transcription factor, NeuroD, as the cell becomes lineage restricted to a neuronal fate. Post-mitotic cells also transiently express prospero-related homeobox gene 1 (Prox 1), an early neuronal-lineage marker, along with DCX. Finally, as the cell matures into a fully-fledged neuron, the early neuronal markers are down-regulated, and the cell expresses neuron-specific nuclear protein (NeuN) [30].

A multitude of intrinsic and extrinsic factors have been shown to regulate each of these stages along the neurogenic pathway, from regulating the maintenance of the stem cell

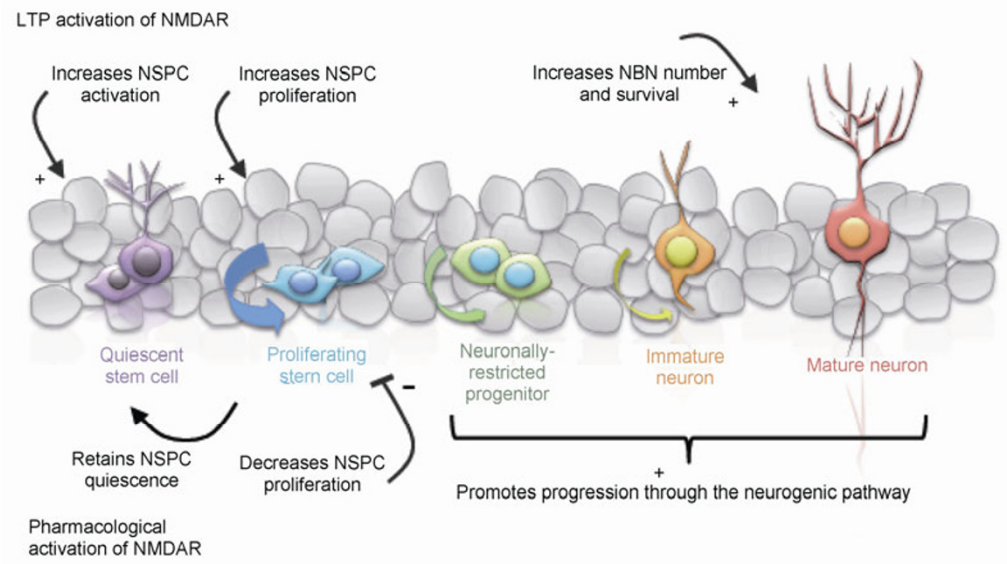

Figure 1 Schematic drawing of the effect NMDAR activation has on neurogenesis in the adult SGZ. Physiological stimulation of the NMDAR, through the induction of LTP, can initiate the proliferation of quiescent NSPCs, as well as enhance the proliferation of active NSPCs. LTP-mediated NMDAR activity has been shown to increase the number of newborn neurons and the survival of immature neurons. Pharmacological manipulations that continuously activate the NMDAR, such as culturing NSPCs in the presence of NMDA, prevent NSPC activation, inhibit NSPC proliferation, and promote cell cycle exit and neuronal differentiation. Abbreviations: LTP, long-term potentiation; NMDAR, *N*-methyl-D-aspartate receptor; NSPC, neural stem and precursor cell; NBN, newborn neuron.

pool to the integration of the maturing neuron [3,25,32,33]. As mentioned above, a number of behaviourally relevant stimuli also regulate adult hippocampal neurogenesis, such as hippocampal-dependent learning paradigms [5], physical activity [6,34], and environmental enrichment [7,8]. Considering that these paradigms are interconnected, such that physical activity and experience in an enriched environment can also improve learning and memory [8,10,35], the underlying mechanism unifying these processes may be synaptic plasticity, as interactive relationships exist between synaptic plasticity, learning, enriched environments, physical exercise and hippocampal neurogenesis.

2 Hippocampal LTP requires the NMDAR

The idea that memories are encoded by the modification of synaptic strength has stood the test of time [36]. Hippocampal LTP provides the experimental evidence to make this initial proposal the field's most compelling cellular model of learning and memory [11]. LTP is easily and reliably induced experimentally by brief periods of high-frequency stimulation (HFS). While LTP has been shown to occur at many sites within the brain, and has been hypothesised to occur at all excitatory synapses [37], this review will focus on LTP in the hippocampus. HFS delivered to the Schaffer/commissural pathway can induce LTP in the CA1 region, while HFS delivered to the perforant pathway can induce LTP in the DG. The induction of LTP in these two areas of the hippocampus is NMDAR-dependent [38], although not all areas of the brain require NMDAR activity for the induction of LTP, for example, the CA3 region of the hippocampus [39].

Functional NMDARs are tetrameric assemblies comprised of two essential GluN1 subunits, and two GluN2 (GluN2A, GluN2B, GluN2C, and GluN2D), and/or GluN3 (GluN3A and GluN3B) subunits [40]. The two non-GluN1 subunits can be identical (di-heteromeric; for example, they could be both GluN2) or different (tri-heteromeric; for example, they could be GluN2 and GluN3) [41]. This large diversity in subunit composition bestows the NMDAR with an equally diverse array of biophysical, pharmacological and signalling characteristics. For instance, the identity of the GluN2 subunit has a substantial influence on the biophysical characteristics of the NMDA channel, such as its conductance, mean open time, and sensitivity to the magnesium ion block [42].

Despite the various NMDAR subunit compositions, all NMDARs have a number of properties that allow them to contribute to fundamental aspects of neural processing, like temporal summation, integration, and plasticity [41]. These properties include the requirement of both glutamate and glycine or D-serine to simultaneously bind to the NMDAR for it to become active. For the NMDAR channel to open, the postsynaptic membrane must be depolarised, to release the magnesium ion that usually blocks the channel during its resting state. Due to these first two properties, the NMDAR is often referred to as a molecular coincidence detector, as it has the ability to associate presynaptic (glutamate release) and postsynaptic (depolarisation) activity [43]. NMDAR channels are also highly permeable to calcium ions, and have unusually slow activation/deactivation kinetics [40,41]. As a consequence of all of these properties, the NMDAR contributes very little to low frequency synaptic transmission [43]. However, when electrical stimuli are delivered to the hippocampus in patterns representing physiological activity associated with learning (theta rhythm), as well as HFS, the NMDAR is activated, and the channel opens to permit calcium entry into the postsynaptic neuron, enabling the induction of LTP [44,45].

The first evidence for the dependence of hippocampal LTP on the NMDAR was published by Collingridge and colleagues [43], who iontophoretically applied the competitive NMDAR antagonist, D-2-amino-5-phosphonovalerate (APV or AP5; also called D-2-amino-5-phosphonopentanoic acid) to the stratum radiatum of CA1, which resulted in a complete block of LTP induction in rat hippocampal slices *in vitro*. They also found that APV did not affect the amplitude of baseline synaptic transmission, nor did it disrupt the maintenance of LTP once induced, indicating that the NMDAR is specifically required for LTP induction [43]. Shortly after this study was published, Harris and colleagues [46] found that the induction of LTP in the CA1 area could be prevented when APV was added to the bath perfusate, but could be induced upon APV washout. Morris and colleagues [47] were the first to show the NMDAR-dependence of LTP in the DG. Following a 6–12 d intraventricular infusion of D-L-APV (but not L-APV), the *in vivo* induction of LTP in the DG was completely abolished [47]. Since these first early studies, many subsequent reports have verified that the induction of LTP in the CA1 and DG is reliant on the NMDAR (reviewed in [11,37]). As mentioned, the NMDAR is highly permeable to calcium ions. Accordingly, the postsynaptic calcium influx that occurs during the opening of the NMDAR is critical for LTP induction. Buffering this postsynaptic calcium rise by the calcium chelator, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetra acetic acid (EGTA) prevents LTP [48]. Downstream of this calcium influx, a number of biochemical second messenger cascades are activated that ultimately lead to the expression of LTP. Antagonising or mutating proteins involved in these cascades impairs the expression and maintenance of hippocampal LTP (e.g., [49–52]).

3 Hippocampal LTP activates NSPC proliferation in the adult hippocampus

It is well documented that neural activity in the DG promotes neurogenesis. A number of studies have shown that

the induction of LTP can activate proliferation in the SGZ in the adult hippocampus. In acutely-implanted anaesthetised rats, LTP induced at perforant path-DG synapses significantly enhanced the proliferation and cell cycle completion of NSPCs (as identified by BrdU incorporation 24 h after HFS) in the SGZ of the DG [9]. The induction of LTP was not found to affect the proliferation of progenitor cells expressing immature neuron markers or the rate of differentiation (as the number of cells double-labelled with BrdU and Prox-1 was similar between the LTP and pseudotetanus groups); however, LTP did significantly enhance the one-month survival of cells that were proliferating 4 d after the HFS [9].

In a similar study, LTP was induced in the DG of chronically-implanted rats, each day for three consecutive days, while BrdU was administered 1 h after each HFS, as well as the day before the LTP induction protocol commenced [53]. The cumulative induction of LTP significantly enhanced the number of proliferating NSPCs in the DG, as measured by BrdU incorporation. Although the time interval between the BrdU administration and animal sacrifice was relatively short, to confirm the LTP-mediated enhancement of proliferation was not due to a modulation of cell survival, Chun and colleagues induced LTP in rats using the 3-d induction protocol, but instead gave a single dose of BrdU 2 h after the last HFS and sacrificed the animals 2 h later. Not surprisingly, LTP significantly increased the number of NSPCs entering the cell cycle, which was confirmed by the expression of the proliferation marker, proliferating cell nuclear antigen (PCNA). These results confirmed that LTP does indeed increase the proliferation of NSPCs [53]. When the competitive NMDA antagonist, 4-(3-phosphonopropyl)-2-piperazine-carboxylic acid (CPP), was administered before each HFS, LTP and the LTP-mediated increase in proliferation was blocked, which indicated the enhancement of proliferation by LTP is dependent on NMDAR activation. Interestingly, in the low-frequency stimulated hemisphere, CPP induced a significant increase in the number of BrdU-labelled cells compared with control-injected rats [53]. The ability of NMDAR antagonists to promote proliferation in the adult SGZ is addressed below.

We recently found that the *in vivo* induction of LTP in the DG of adult mice significantly and specifically increased activation of the quiescent NSPC population in the SGZ *in vitro* [12]. Using the neurosphere assay, in which actively proliferating NSPCs form a ball of cells called a neurosphere, a significantly greater number of neurospheres was obtained following the successful induction of long-lasting late-LTP, but not following electrophysiological stimulation that failed to induce LTP, or only induced a short-lasting early-LTP. These results suggest that LTP activates the quiescent NSPC population, as more NSPCs were stimulated to form neurospheres [12,23]. Furthermore, the administration of CPP to mice before the HFS prevented LTP and the LTP-mediated enhancement of NSPC activation. It is intriguing that the magnitude of LTP was found to be strongly correlated with the extent of precursor cell proliferation, suggesting a dose-response-like relationship between synaptic strengthening and activation of proliferation [12].

The mechanisms underlying how the induction of LTP induces an increase in NSPC activation and proliferation have not yet been definitively revealed. While speculative, it is possible that LTP primes the NSPCs to respond to factors that directly activate proliferation, via paracrine signalling mechanisms. LTP has been shown to initiate the release of many neurotrophins (e.g., brain-derived neurotrophic factor (BDNF), serotonin, norepinephrine, dopamine, and nitric oxide synthase) [54,55] that are known to modulate adult hippocampal neurogenesis [56,57], and could mediate the observed increase in proliferation following LTP. A paracrine signalling mechanism has been proposed to explain the activity-dependent enhancement of neuronal differentiation in a co-culture model made from EGFP-labelled adult NSPCs with neurons and astrocytes isolated from postnatal day 0 (P0) and P1 mice [58]. Exposure of the co-cultures to a glycine-enriched magnesium-free medium (to activate NMDARs) induced intracellular calcium oscillations, increased mini excitatory postsynaptic currents (mEPSCs) and enhanced neuronal differentiation of the NSPCs. When the precursor cells were cultured in isolation (without the neurons and astrocytes) and exposed to glycine, no change in neuronal phenotype was observed. This effect was found to be NMDAR-dependent and mediated by the activity-dependent release of BDNF from mature neurons present in the co-culture system [58]. As such, NMDA-mediated network activity can influence neuronal differentiation via signalling between mature neurons and NSPCs, so it is feasible that a similar mechanism may underlie the NMDAR-mediated network activity influencing the activation of proliferation.

NMDAR-mediated neural activity has also been shown to modulate other steps along the neurogenic pathway, such as the survival and integration of newborn neurons. For instance, using a retroviral gene knockout technique to specifically remove the NMDAR GluN1 subunit from dividing NPSCs in the hippocampus, Tashiro and colleagues [59] were able to show that newborn neurons lacking functional NMDARs were significantly more vulnerable to cell death 2–3 weeks after birth, indicating that NMDARs mediate an input-dependent modulation of newborn cell survival. The induction of LTP in the DG of awake and freely moving rats has been shown to promote the survival of neurons born 7–10 d prior to the HFS (but not younger or older neurons), without affecting the degree of neuronal differentiation [13]. NMDAR-mediated neural activity has also been shown to modulate the spinogenesis and integration of newborn neurons into the pre-existing DG hippocampal circuitry [60]. In agreement with previous literature, the ability of LTP to enhance spinogenesis and integration appears to depend on

the developmental stage of the newborn neuron [60].

4 Direct modulation of NMDARs alters NSPC proliferation in the adult hippocampus

As mentioned above, activity-dependent synaptic plasticity, enriched environments and hippocampal-dependent learning tasks all activate and enhance the proliferation of NSPCs in the adult hippocampus. Antagonising the NMDAR during this neural activity has been shown to prevent the observed increase in proliferation, highlighting the importance of the NMDAR in this process. To further identify the NMDAR as an important modulator of NSPC proliferation, several research groups have pharmacologically targeted the NMDAR using antagonists and agonists, to assess what impact these manipulations have on proliferation *in vivo*. Cameron and colleagues [61] were the first to show that NMDAR activity regulates the proliferation of NSPCs in the adult hippocampus. Adult rats were injected the proliferation marker, ^{3}H-thymidine, 1 h after the systemic administration of the competitive NMDAR antagonist, CGP43487; the non-competitive antagonist, MK801; or saline. After sacrifice, Cameron and colleagues found that both the CGP43487 and MK801 treatments had induced a 2-fold increase in the number of ^{3}H-thymidine-labelled cells in the SGZ, compared to the control group. In contrast, treatment with NMDA, the major agonist of the NMDAR, resulted in a significant reduction in proliferation in the SGZ [61]. Kitayama and colleagues [62] repeated these findings using a slightly different BrdU protocol. They also found that while the systemic NMDA administration significantly reduced proliferation and NSPC number, it did not induce neuronal death, indicating that activation of the NMDAR specifically decreased proliferation. The pro-proliferative effect of blocking the NMDAR has been shown to persist for at least a week [63]. Comparable findings have been reported using aged rats [64]. As mentioned above, NMDARs in the hippocampus are primarily composed of GluN1 and GluN2A or GluN2B subunits. Hu and colleagues [65] addressed which subunit may be mediating the pro-proliferative effect of NMDAR antagonism, by treating adult mice with the specific GluN2B antagonist, Ro25-6981. Significantly more BrdU-labelled cells were present in the DG of Ro25-6981 treated animals, compared to saline treated controls, suggesting that under normal conditions the GluN2B subunit negatively regulates hippocampal proliferation, possibly through a nitric oxide synthase-mediated pathway [65]. These studies provide evidence that the NMDAR specifically modulates NSPC proliferation *in vivo*.

Nevertheless, not all reports support the hypothesis that NMDAR antagonism increases NSPC proliferation *in vivo*. Joo and colleagues [66] have shown that while NSPC proliferation is increased in the SGZ 3 days after MK801 treatment, and is decreased after NMDA treatment, the opposite results were observed 28 d after treatment—MK801 reduced the number of BrdU-labelled cells while NMDA increased BrdU numbers, indicating that activation of the NMDAR had increased the survival of proliferating cells [66].

To ascertain the identity of the cells within the neurogenic pathway that proliferate in response to NMDAR blockade, Petrus and colleagues [67] utilised adult mice expressing GFP under the nestin promoter, as well as immunohistochemical markers of stem cell identity. One hour following MK801 treatment, more NSPCs had entered the cell cycle, identified by nestin-GFP expression and BrdU incorporation, while the number of proliferating progenitor cells expressing nestin and DCX did not change compared to saline controls. Twenty-four hours after MK801 treatment, a greater number of late-progenitor cells expressing DCX were proliferating. At 4 weeks, the number of BrdU-labelled cells that retained GFP was reduced, while the number of BrdU-positive cells co-labelled with NeuN had increased. These results suggest that NMDAR antagonism promotes the proliferation of stem cells, stimulates their progression through the neurogenic pathway, and increases neuronal differentiation at the expense of the NSPC population [67].

In the studies reviewed above, NMDAR function was manipulated using pharmacological agents. An alternative approach to alter the function of the NMDAR has been to use genetic manipulation. As discussed previously, the GluN1 subunit is a requirement for NMDAR activation, and genetic knockout of GluN1 renders the NMDAR completely non-functional. Unfortunately, complete removal of the GluN1 subunit results in the perinatal death of the mouse. To avoid this problem, Bursztajn and colleagues [68] utilised heterozygous mice that have only one copy of GluN1, to decrease the expression of functional NMDARs. Partial inactivation of the NMDAR significantly increased NSPC proliferation in the SGZ of the adult hippocampus, without altering the differentiation phenotype of the newborn cells [68]. In 3-week-old transgenic mice lacking the GluN2A subunit of the NMDAR, basal proliferation and survival of newborn cells in the hippocampus was similar to wild-type mice [69]. However, the running-induced enhancement of NSPC proliferation observed in wild-type mice was suppressed in the GluN2A knockouts, demonstrating that GluN2A receptors may mediate the activation of proliferation, possibly through BDNF [69]. These genetic approaches to modify NMDAR function circumvent the potential side effects of systemic pharmacological manipulations, and provide further evidence that the NMDAR regulates NSPC proliferation *in vivo*.

Some studies have taken a reductionist approach, and have assessed the NMDAR-mediated mechanisms of proliferation *in vitro*. When NSPCs isolated from young adult mice were cultured in the sustained presence of NMDA, the formation of neurospheres was markedly inhibited. When

these neurospheres were transferred to a differentiation-promoting medium (containing all-*trans* retinoic acid, ATRA), the prior NMDA treatment promoted commitment to a neuronal fate [70]. Deisseroth and colleagues [71] have also investigated whether manipulations that increase NMDA-mediated neuronal activity can influence NSPC differentiation *in vitro*. NSPCs isolated from the adult hippocampus were co-cultured on a substrate of ethanol-fixed P0 hippocampal cells, in media permitting differentiation (i.e., tapered mitogen levels). Upon excitation, via a high potassium concentration to depolarise the cells, or glutamate to activate the NMDAR, significantly more NSPCs differentiated into MAP2-positive neurons. Treatment of the co-culture with APV inhibited the development of neurons, both under basal conditions and with excitation, while NMDA treatment facilitated neuronal differentiation [71].

While these *in vitro* studies show that NMDAR activity can reduce the proliferation and promote the neuronal differentiation of adult-derived NSPCs, conflicting results have been found in studies using embryonic or early postnatal cells. For instance, Joo and colleagues [66] have shown that proliferation is positively regulated by NMDAR activity in the embryonic hippocampus, and similar results have been shown in the P0 hippocampus [72]. On the other hand, treatment of P0 cultures with Ro 25-6981 or MK801 can significantly decrease neurosphere formation [72].

These studies highlight the importance of the NMDAR in modulating the proliferation and differentiation of NSPCs, both *in vivo* and *in vitro*. Pharmacological blockade of the NMDAR and genetic knockdown of GluN subunits promotes the proliferation of NSPCs, with some reports showing an increase in the number of NSPCs. On the other hand, the sustained activation of the NMDAR reduces the proliferation of adult-derived NSPCs and facilitates their development through the neurogenic pathway, favouring neuronal differentiation (Figure 1).

5 NMDAR subunit expression on NSPCs

To determine whether NMDAR manipulations are likely to act directly on the NSPCs, or indirectly via mature granule cell (or astrocyte) modulation, a few groups have addressed whether NMDAR subunits are actually present on adult hippocampal NSPCs. Adult hippocampal newborn neurons, labelled with either BrdU or an intrahippocampal injection of a GFP retroviral construct, were assessed at various time-points for the presence of NMDAR subunits using immunohistochemistry [73]. Most cells expressing GFAP displayed GluN1 and GluN2B immunoreactivity; however, these cells were not co-labelled with other stem cell-specific markers to distinguish them from astrocytes. Most very young BrdU-labelled cells (2- and 24-hour-old) did not display detectable levels of GluN1 and GluN2B. Nevertheless, about 20% of these very young cells did show some weak staining. In late progenitor cells with clear expression of NeuroD, very little GluN1 and GluN2B expression was observed; however, in immature neurons with faint NeuroD expression, more intense GluN1 and GluN2B were detected. By 2 weeks of age, clear GluN1 and GluN2B staining was observed in most BrdU-labelled newborn neurons, and cells co-stained with DCX showed a similar pattern of GluN1 and GluN2B expression [73]. In another study, Petrus and colleagues did not find any GluN1 immunoreactivity in nestin-GFP positive NSPCs in the SGZ of adult mice [67]. As these *in vivo* studies report mixed results as to whether NSPCs express NMDARs, further investigation is warranted, given the strong evidence that NMDARs modulate NSPC proliferation.

NMDAR subunit expression in NSPCs isolated from the adult rodent hippocampus has also been investigated. Using neurospheres cultivated from NSPCs, Kitayama and colleagues [70] performed real-time polymerase chain reaction (RT-PCR) to detect NMDAR subunit mRNA, and immunohistochemistry to label NMDAR subunit proteins. Cells showed marked GluN1 subunit mRNA levels throughout the culturing period of 2–10 d, which peaked and plateaued at day 6. In contrast, GluN2D mRNA expression was high at the start of the culturing period and progressively declined over time. GluN1, GluN2A and GluN2B expression was detected by immunohistochemistry from the fourth day *in vitro*. To confirm the GluN subunits formed functional NMDARs, 12-day-old neurospheres were briefly exposed to NMDA, which induced the marked expression of activity-dependent Jun and Fos family transcription factors, in an MK801-sensitive manner [70]. In another study, NSPCs isolated from the P0 rat hippocampus were cultured for 5 d, and shown to express GluN1 and GluN2B mRNA (as determined by RT-PCR and Western blotting), as well as GluN1 and GluN2B proteins (visualised by immunohistochemistry) [72]. Finally, NSPCs isolated from the adult rat hippocampus co-cultured on a substrate of ethanol-fixed P0 rat hippocampal cells (so the only viable cells present are the NSPCs) responded directly to excitatory stimuli by showing an NMDAR-dependent increase in intracellular calcium concentration [71]. Data from the *in vitro* studies indicate that cells within neurospheres generated from young and adult NSPCs express NMDAR subunits. It must be noted, however, that after several days *in vitro*, cells present in a neurosphere generated from NSPC proliferation can spontaneously differentiate, even in the presence of mitogens (C Taylor, unpublished data, 2013), so it is possible that some of the cells expressing NMDAR subunits in these studies were not strictly NSPCs, and had begun to mature from their neural stem/precursor state into early neurons or glia. Nevertheless, NSPCs can respond directly to manipulations that are blocked by NMDAR antagonists [70,71], suggesting that functional NMDARs are also present on hippocampal NSPCs.

6 Summary

It has long been known that hippocampal-dependent learning tasks, physical activity and enriched environments stimulate NSPC proliferation in the adult hippocampus [5–8,10]. We have reviewed the evidence showing that NMDAR-dependent synaptic plasticity in the hippocampus promotes the activation and proliferation of NSPCs in the adult SGZ (Figure 1). On the other hand, specific inhibition of the NMDAR, both *in vivo* and *in vitro*, enhances the proliferation of hippocampal NSPCs, while direct activation of the NMDAR reduces proliferation and promotes cell cycle exit and neuronal differentiation (Figure 1). Nonetheless, what needs to be resolved is how synaptic plasticity-mediated activation of the NMDAR promotes proliferation, while the activation of NMDARs with NMDA treatment inhibits proliferation.

It is possible that different patterns of NMDAR activation may trigger different mechanisms of regulation. We believe that the induction of LTP may prime NSPCs to positively respond to paracrine factors that promote proliferation. For instance, the induction of LTP is known to promote a cascade of postsynaptic events, including the release of a number of pro-proliferative factors (see above) that may act on NSPCs to activate and increase their proliferation. As such, activation of the NMDAR with physiologically relevant patterns of neural activity is likely to elicit a different effect on the NSPCs than prolonged activation of the NMDAR by direct NMDA treatment. Sustained NMDAR activation is unlikely to initiate the release of pro-proliferative factors observed following the induction of LTP. Instead, continuous activation of the NMDAR is more likely to shut down actively proliferating stem cells to protect them from NMDAR-mediated excitotoxicity. Indeed, some *in vitro* studies have used sustained concentrations of NMDA that were shown to cause excitotoxic death of the NSPCs (e.g., [66]), so it is not surprising that proliferation of NSPCs can be inhibited under these conditions. Nevertheless, other studies report that cell death is not induced by the NMDA treatment (e.g., [62]).

It is also possible that different NSPC subtypes are attuned to different patterns of NMDAR activation. For instance, we have shown that *in vivo* electrophysiological stimulation that induces LTP in an NMDA-dependent manner specifically targets the quiescent stem cell population to start proliferating *in vitro* [12]. On the other hand, more persistent activation of the NMDAR, by NMDA application or NMDA-mediated excitatory stimuli (high potassium and glutamate), appears to target actively proliferating NSPCs to exit the cell cycle and differentiate into neurons [70,71]. In contrast to sustained NMDAR activation, conditions in which antagonists block the NMDAR, or the NMDAR function is reduced by genetic manipulation, the NSPCs may become stuck within the cell cycle, and over-proliferate.

Nonetheless, calcium influx through the NMDAR is most likely to mediate its ability to modulate NSPC proliferation. Calcium influx through voltage-gated calcium channels (VGCCs) has also been shown to mediate depolarisation-induced activation of the quiescent NSPC population [23], and enhance neuronal differentiation of the progenitor cell population [71]. NMDAR-mediated stimulation of adult hippocampal NSPCs has been shown to rapidly induce the expression of the pro-neuronal transcription factor, NeuroD, and down-regulate the expression of anti-neuronal phenotype genes, indicating that signalling through NSPC NMDARs can directly control the fate of NSPCs undergoing differentiation [71]. Calcium signalling through the NMDAR may also modulate proliferation in a similar manner, but is yet to be shown.

In conclusion, it is evident that the NMDAR is clearly involved in the modulation of NSPC activation and proliferation, both *in vivo* and *in vitro*. Understanding the precise mechanisms by which the NMDAR controls the progression of NSPCs through the neurogenic pathway will enable us to address a new avenue of NSPC manipulation, in order to harness the therapeutic potential of endogenous neurogenesis in the adult brain, and facilitate recovery from neurodegeneration and psychiatric illness [24]. Already, a number of studies have investigated how the moderate-affinity NMDAR antagonist, memantine (which is used in the clinical treatment of Alzheimer's disease), promotes the proliferation of NSPCs in the adult hippocampus [74–76].

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